

Chicken Farm Biomarker Progress Report 8-2-06

- Develop strain specific PCR primers for those candidate organisms common to litter and soils
 - look for variable regions among the top 20 BLAST sequences that closely align or those >95% similar
 - compare primers to RDPII database to determine what else they might amplify
 - look for thermodynamic/folding problems with the primers
 - subject below is Northwind's sequence, query is Pantoea ananatis (below):

gi|72384803|gb|DQ133545.1| Pantoea ananatis strain BD 543 16S ribosomal RNA gene, partial sequence Length=1533
 Score = 1346 bits (679), Expect = 0.0 Identities = 694/699 (99%), Gaps = 0/699 (0%) Strand=Plus/Minus

Green = primer sequence

Query 49	TCTCTACGCA	TTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCAAGCCTG	108
Sbjct 699G.....	640
Query 109	CCAGTTCAAATACAGT	CCCAGGTAAAGCCGGGGATTCACATCTGACTAACAGACC	168
Sbjct 639G.....	580
Query 169	GCCTGCGTGCGCTT	TACGCCAGTAATTCCGATTAAACGCTTGACCCCTCCGTATTACCGC	228
Sbjct 579	520
Query 229	GGCTGCTGGCACGGAGT	TAGCCGGTCTTCTGCGGGTAACGTCAATCGATGCGGTTA	288
Sbjct 519C.....	460
Query 289	TTAACACATCGCCT	CCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCCTTCATA	348
Sbjct 459	400
Query 349	CACGCCGCATGGCTGCATCAGGCT	TGCGCCCATGTGCAATATCCCCACTGCTGCC	408
Sbjct 399	340
Query 409	CGTAGGAGTCTGGACC	GCGTCTCAGTGTGGCTGGTCATCCTCTCAGACCAGCTA	468
Sbjct 339	280
Query 469	CGGATCGTCGCCTAGGT	GGGCCATTACCCGCCTACTAGCTAATCCCATCTGGGTTCATC	528
Sbjct 279	220
Query 529	CGATAGTGAGAGGCC	GAAGGTCCCCCTTTGGCTTGCGACGTTATGCGGTATTAGCC	588
Sbjct 219	160
Query 589	ACCGTTCCAGTGGT	TATCCCCCTCTATCGGGCAGATCCCCAGACATTACTCACCCGTCC	648
Sbjct 159	100
Query 649	GCCACTCGTACCCA	AAGGAGCAAGCTCTGTGCTACCGTCCGACTTGCATGTGTTAGG	708
Sbjct 99G.....	40
Query 709	CCTGCCGCCAGCGTT	CAATCTGAGCCATGATCAAACCTCT 747	
Sbjct 39G..... 1	

Results

1. Primers work for all 4 biomarkers from the clone library
2. Still testing the primers against the original soil and litter samples
 - a. E. coli strain specific primer is working in all the soil and litter samples
 - b. Optimizing PCR for the 3 other primers in the soil and litter samples

Fragment occurs in the following replicates. Underline indicates that it represented <1% of community in that replicate

E.Coli MSP fragment	Litter A	Litter B	Soil A	Soil B
496.0	1,2,4,5	1,2,3,5	1,2,4	all
498.9	all	all	1,2,4,5	all
500.8	all	all	all	all

Universal MSP fragment	Litter A	Litter B	Soil A	Soil B
80.1	1,2,3,4	all 5	none	1,3,4
130.9	1,3,4	all 5	3	none
142.9	all	1,2,3,4	1,4	1
147.3	all	all 5	all	1,4
158.9	2,3,4	all 5	2,3,4,5	1,4
165	1,3,4	all 5	1,3,4,5	1,4

Next Steps

- Optimize PCR for environmental samples while testing primer specificity against the original soil and litter samples
- Test PCR primers against other fecal samples from the impacted watershed
- Test PCR primers against the available closely related clones or cultures from researchers or culture collections
 - have in hand or access to the specific strain named in the NCBI database for 3 of the 4 biomarkers—and one closely related strain for the last biomarker

Details on primer specificity

Clone/ T-RF/Organism	Forward Primer Matched in RPDII	Reverse Primer Matched in RPDII
Clone SA19 T-RF 158.9 Kineococcus	AY919955, AY426452, AF195447, AF513961, AY862810, AM085954, AM182287, AM182297, AM182298, AM182299	0
Clone SB37 T-RF 142.9 Uncultured organisms	0	0
Clone LA35 T-RF 147.3 Brevibacterium	0	0
Clone SA15 T-RF 500.8 Pantoea ananatis	0	AJ010486, DQ221344, AF364845, AF364844, AY528223, AY579209, AY579211, U80196, U80209, AB004758, AB027693, AY530796, AJ629190, AB178169, AB178170, AY898643, AB114622, DQ133548, DQ195524, AB242937, AB242945, AB242946, AB242979 30 sequences in Enterobacter